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(54) Title: PROCESS FOR LACTOSE HYDROLYSIS IN MILK AND OTHER DAIRY PRODUCTS USING SONICATED DAIRY CULTURES

(57) Abstract

A composition useful for enzymatically hydrolysing lactose in dairy products. The composition comprises a sonicated culture medium containing bacterial cells which produce lactase within the cells during culture. The bacterial cells are non-toxic to humans and compatible with dairy products. The bacterial cells are cultured and then ruptured by sonication to release lactase into the culture. The culture media may then be introduced to milk or other dairy products and incubated for a predetermined period of time to hydrolyse lactose.

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PROCESS FOR LACTOSE HYDROLYSIS IN MILK AND OTHER DAIRY PRODUCTS USING SONICATED DAIRY CULTURES

FIELD OF THE INVENTION

This invention relates to the use of lactase derived from microorganisms for purposes of hydrolysis of lactose in dairy related products and in particular in milk.

Background of the Invention

Lactose in dairy products presents both a processing problem in concentrating milk and the manufacture of cheese as well as a health problem for people with lactose intolerance. Considerable work has therefore been done on the conversion of lactose in dairy products to a combination of simple sugars, which are more readily processed and are also more readily digested by lactose intolerant people. The lactase enzyme, which hydrolyses lactose, is commercially available and is normally manufactured by the culturing of lactase producing microorganisms, such as bacteria, yeast or moulds. After culturing of the microorganisms for a suitable period of time the microorganisms are treated to isolate the lactase from the microorganisms for purification of the lactase and use of purified lactase in hydrolysing lactose in dairy products.

United States Patent 2,681,858 discloses the culturing of several types of bacteria, including, lactobacillus and bulgaricus. To produce lactase, great care has to be exercised in isolating the lactase from the microorganisms so as to avoid contamination of the lactase and to ensure the lactase is not denatured in the separation process. It has been thought for some time that contaminating protein and other cell constituents may have an adverse effect on the conversion of lactose in dairy products. It has also been understood for some time that the cell constituents would also alter significantly the taste of the product, such as,

substantially reducing the pH of the product and also making the dairy product bitter.

United States Patent 4,007,283 suggests the radical rupture of cell membranes to remove lactase from the cultured microorganisms. After cell rupture, usually by mechanical techniques, the enzyme is then purified as extracted from the culture and used in normal way.

United States Patent 4,234,687 discloses the splitting opening of cells to release lactase from the bacterial cells. The debris of cell wall and the like in the culture is separated from the lactose, the lactase is then introduced to milk to hydrolyse the lactose.

United States Patent 4,332,895 disclosed the use of immobilized whole cells for the hydrolysis of lactose. The whole cells are immobilized in a gel, lactase is released from the whole cells to hydrolyse lactose in whey and milk.

Summary of the Invention

We have discovered that lactase producing bacteria may be treated by sonication techniques to release lactase into the bacterial culture material. Such sonicated culture material may then be introduced directly into milk, whey or other dairy products to hydrolyse lactose. According to another aspect of our discovery, the bacterial cells may be introduced to the dairy product and sonicated in situ to release lactase into the dairy product for hydrolysis of lactose. We have found that such techniques effectively hydrolyse the lactose in the dairy products without affecting colour, smell, or taste of the dairy product.

In accordance with an aspect of the invention, a composition useful for enzymatically hydrolysing lactase in dairy products, the composition comprises a sonicated culture medium containing micr bial cells which produce lactase within the cells during culture wherein:

the cells are non-toxic to humans and compatible with dairy products;

sonication of the cells ruptures the cells to release thereby said lactase into the culture, and

the culture media containing ruptured cell wall material, any remaining whole cell material after sonication and contents of said ruptured cells.

According to a further aspect of the invention, a process for enzymatically hydrolysing lactose in dairy products comprises the use of the above composition. The culture medium, as treated, is introduced into the dairy product and incubated for a sufficient period of time to hydrolyse lactose to an acceptable degree.

According to another aspect of the invention, a process for enzymatically hydrolysing lactose in dairy products comprises adding the culture of bacterial cells to the dairy product and sonicating the dairy product to release in situ the bacterial cell content. The system is then incubated at a temperature in the range of 55°C to hydrolyse lactose in the dairy product.

According to another aspect of the invention, the above composition can be prepared by culturing the bacterial cells in a suitable culture medium to produce lactose within the cells and continuing culture of the cells until lactose concentration is at a maximum for harvest. The culture medium is then sonicated to rupture a majority of the cells to release thereby the lactase into the culture medium.

According to another aspect of the invention, the bacterial cells employed in the process are of a species which, when treated by sonication to release cell contents into the culture medium and the medium is introduced to the dairy product, and the system incubated at a temperature in the range of 50° to 60°C, the lactase as released from the cells retains its lactose hydrolysing activity while other enzymes and proteins released from the contents of the bacterial cells do not affect dairy product quality due to neutralization or inactivation at the higher incubation temperatures.

Brief Description of the Drawings

Preferred embodiments of the invention are shown in the drawings wherein:

Figure 1 is a graph showing relationship of enzyme activity versus pH of culture during sonication of cells in culture; and

Figure 2 is a graph showing relationship of enzyme activity versus temperature of culture during sonication of cells in culture.

Detailed Description of the Preferred Embodiments

Applicant's discovery leads to a more economical process for accomplishing lactose hydrolysis in dairy systems, products and the like. The process has been developed in a manner so as to have no regulatory or legal limitation because of its use of dairy microorganisms with a long history of industrial use and hence, safe for consumption. On an industrial scale, lactose hydrolysed milk is used in the manufacture of fermented dairy products, such as, cottage cheese, buttermilk, yogurt, and the like. There is a considerable shortening of the fermentation process and other process advantages realised in hydrolysing lactose in the milk before treatment. However, the current high costs of enzymatic lactose hydrolysis preclude these potentially beneficial industrial uses. Such current industrial hydrolysis techniques involve the use of highly purified enzyme preparations obtained from yeast or fungal sources, and used in a free or immobilized form. For example, the lactase enzyme may be immobilized on a resin where the dairy products are passed through the resins to achieve chemical hydrolysis of lactose in the dairy product. It is preferable to carry out such reactions at high temperature and low pH which is not practical for treatment of foods due to major side ffects.

According to the process of this invention, ordinary dairy type microbial cultur s may be grown to

produce lactase. As is appreciated there are a variety of techniques for optimizing the culture of such microorganisms. At the time of optimum production of the lactase, the culture is subjected to a sonication treatment to rupture the cells and thereby release into the culture medium, the bacteria or yeast cell contents which includes lactase. The sonication treatment ensures that, for example, the bacterial culture material contains low levels or undetectable levels of viable bacterial cells, but surprisingly retains high enzymatic activity for breaking down the lactose in milk and other dairy products. We have discovered that the sonicated culture can be added directly to milk, whey or other dairy products to hydrolyse lactose without any side effects. By use of proven food grade microorganisms to produce the lactase, it may be safely added to the dairy products in view of their long history of safe application in industry, without any of the need for further regulatory approval.

We have also found that due to the low viability of any whole cells remaining in the sonicated culture there is little, if any, detectable subsequent fermentation activity in the treated dairy product, so that the dairy liquid can be use in its normal manner. For example, milk treated with the sonicated culture can be marketed as a lactose reduced product, without the need for any further treatment to remove the sonicated bacterial culture.

The microorganisms selected for use in accordance with this invention, are those with high levels of intracellular lactase enzyme. There are several strains available which produce high levels of lactase. For example, strains belonging to the following genera:

Bacteria
Lactococcus
Lactobacillus
Leuconostoc
Streptococcus
Bifidobacterium
Propionibacterium
Pediococcus

and such other genera possessing the ability to ferment lactose. It is appreciated that this list contains hundreds of individual species and strains, most of which are used by the dairy industry.

Yeasts: there are several types of yeasts that also ferment lactose including the following genera:

Candida (e.g. *Candida kefir)*
Kluyveromyces (e.g. *K. marxianus)*
Sacharomyces

which are also used in the dairy industry.

The sonicated culture may be preserved in variety of manners in accordance with techniques routinely employed by those skilled in the art. Selected organisms are grown in a suitable culture medium until optimum enzyme production is achieved. Optimum, pH and temperature conditions may be employed in the production of the crude enzyme. The cultures are subjected to sonication at a suitable frequency, for example, in the range of 16 KHz. The period of sonication is selected to ensure that most, if not all cells are ruptured to release the lactase enzyme. As aforementioned, the treated culture is then added to the dairy product for purposes of hydrolysing the lactose. As also discussed it is appreciated that the microorganisms may be added to the dairy product and then sonicated to release in situ enzyme into the dairy product for purposes of lactose hydrolysis.

According to a preferred aspect of th invention, microorganisms of th above list may be

selected which produce lactase and which, when the lactase is released from the microorganisms and used to hydrolyse lactose in milk, can withstand higher incubation temperatures, preferably in the range of 50° to 60°C. At these higher incubation temperatures, it has been found that the lactase retains its hydrolysing activity while other proteins and enzymes, as contents of the ruptured cells and which normally have an impact on milk quality by either reducing its pH and/or adding to its bitterness and other unsuitable characteristics, are neutralized and/or denatured. A particularly useful species in this regard is *L. delbrueckii subsp. bulgaricus*, the properties of which are investigated in the following examples. The bacteria may be cultured at a suitable temperature and pH to optimize production of the lactase. The lactase may then be harvested by sonicating the bacteria to rupture a majority of the bacteria and thereby release lactase into the culture media. It has been found that incubation of the dairy product with the released lactose in the culture medium of this bacteria actively hydrolyses lactose to levels in the range of 75% without appreciably affecting the quality of the dairy product. This is presumed to be due to inactivation at these higher temperatures of other enzymes and proteins released into the medium during rupture of the cells. It has also been surprisingly found that incubation of the culture medium in the dairy product does not appreciably reduce pH and hence does not impact on the quality of the dairy product. It has always been thought that contents of the rupture bacterial cells would release other enzymes and proteins into the dairy product which would appreciably affect the taste and/or other qualities of the product, particularly milk. As demonstrated in the following examples, this has be n found not to be the case and hence a significant benefit to this process.

The following examples demonstrate the sonication of various cultures and the optimization of the culture conditions:

Example 1: **Propagation of cultures**

Pure cultures of *S. thermophilus and L. delbrueckii subsp. bulgaricus* were isolated from a commercial yogurt sample. The isolated cultures were examined for purity by conventional methods (Hardie et al, 1986. In "Bergey's Manual of Determinative Bacteriology," *The Williams and Wilkins Co., Baltimore, MD.*). Freeze-dried culture of *L. acidophilus* was obtained from Dept. of Microbiology, North Carolina State University.

Each culture was maintained in sterile 12%(w/v) reconstituted non-fat dry milk (NDM) as well as Difco All-purpose Tween (ATP) broth. Sterile 100 mL batches of NDM or APT broth were inoculated with 1% of each culture and incubated at 43°C for *L. delbrueckii subsp. bulgaricus*, 37°C for *S. thermophilus and L. acidophilus. These incubation temperatures were used throughout this study, unless indicated otherwise. Cultures were transferred successively at least three times before use.*

Example 2: ***Effect of sonication on release of β-galactosidase.***

Ten grams of each culture were mixed with distilled water, blended for 1 minute and the final volume was made to 100 mL in a volumetric flask. Aliquots of the diluted sample held in an ice-bath were sonicated for 20 minutes for L. acidophilus culture and for 10 minutes for other cultures using Sonic 300 dismembrator (Artek Systems Corp., Farmingdale, NY 11735) at frequency of 16 KHz. Samples were taken every minute and 1 mL portions of the sonicated solution were use to determine enzyme activity. The temperature of the sample was also checked every minute and sampl temperature adjusted downwardly by cooling as required to avoid a ris in temperature during sonication. In this

arrangement, the sample temperature during sonication did not exceed 20°C.

Example 3: **Assay for β-galactosidase (lactase)**

To determine the enzyme activity, 10g of each sonicated or unsonicated culture were mixed with distilled water, blended for 1 minute and the final volume was made to 100mL in a volumetric flask. One mL of the solution was used the in assay, carried out according to Citti et al. (1965). Solutions of 0.005M o-nitrophenyl-β-D-galactopyranoside (ONPG) substrate were prepared in 0.1M phosphate buffer, pH 7.0, and 1 mL aliquots of the diluted sonicated culture samples were incubated with 5 mL ONPG solution for 15 minutes at 37°C. The reaction was stopped by adding 2.5 mL 1M cold sodium carbonate. The amount of o-nitrophenol released by the enzyme action of the substrate was measured with a Spectronic 21 spectrophotometer (Bausch and Lomb Inc., Rochester, NY) at 420 nm. The unit of lactase activity was estimated according to the method of Mahoney et al. (1975). "Selection of strain, growth conditions, and extraction procedures for optimum production of lactase from *Kluyveromyces fragilis*." *J. Dairy Sci.* as the amount of the enzyme which liberated one μmole o-nitrophenol from ONPG per min gram samples 37°C. Chemicals were obtained from Sigma (P.O. Box 14508, St. Louis, Mo 63178).

Example 4: **Production of lactase in broth systems**

The organisms grown in the APT broth were routinely propagated and transferred successively three times, then the active cultures were transferred to Lactobacilli MRS broth (Difco Laboratories, Detroit, Michigan) or Difco APT broth containing either 0.01 g/mL glucose (GAPT) or 0.01 g/mL lactose (LAPT). The cultures were grown for 18 hr. At the end of the incubation period, cultures were immediately chilled and centrifuged at 16300 x g for 10 min at 1°C in a Sorvall Model RC-5B (Du Pont Co., Diagnostic and Bioresearch Systems,

Wilmington, DE) superspeed centrifuge. The harvested cells were washed by dissolving in 100mL distilled water, recentrifuge, and suspended in 20 mL distilled water for sonication in two portions. One portion was used in the β-gal assay which represented the total enzyme activity. The other portions were centrifuged at 13100 x g for 10 min to remove the cell debris. The supernatant liquid was also assay for β-gal to estimate the free enzyme which was not bound to the cell wall. The difference between the two assays represented the enzyme bound to the cell wall. The cell debris were dried at 105°C for 2.5 hr to obtain the dry weight of cell suspensions.

Example 5: **Properties of β-galactosidase**

To determine the optimum pH and temperature conditions of the crude enzyme preparations, the enzyme isolated from acetone precipitation was diluted 1500 times in distilled water for *L. delbrueckii subsp. bulgaricus and S. thermophilus* lactases, and 250 times for *L. acidophilus* lactase. The optimum pH of the enzyme was determined by measuring enzyme activity in phosphate buffer at 37°C over a pH range of 4.5-7.5 (8.5 in case of *S.thermophilus* lactase). The optimums for pH, as shown in Figure 1, are in the range of 6 to 7. Different proportions of 0.2M mono- and disodium phosphate buffer were used to obtain the desired pH. The optimum temperature for enzyme activity was then determined by measuring enzyme activity at the optimum pH over a temperature range of 35-65°C. The optimums for temperature, as shown in Figure 2, are in the range of 55°C.

Example 6: **Lactase activity and properties of sonicated dairy cultures**

After determining lactase activity of 2 strains of Lactococcus lactis subsp. cremoris and 2 strains of *Lactobacillus delbrueckii subsp. bulgaricus* 11842, the properties of crude lactase isolated from *L. delbrueckii subsp. bulgaricus* 11842 were studied to ascertain whether

sonicat d dairy cultures can be used for lactose hydrolysis in milk.

The enzyme activity was determined by incubating the sonicated cultures with o-nitro-phenyl-beta-D--galactopyranoside (ONPG) or o-nitrophenyl-beta-D-galactopyranoside-6-phosphate (ONPG-6P) and measuring the amount of o-nitrophenol released. Crude enzyme extract from *L. delbrueckii subsp. bulgaricus* cultures of *L. delbrueckii subsp. bulgaricus* 11842 were subjected to sonication at pH 7.0. The sonicated culture was incubated with autoclaved milk at 55°C and percent lactose hydrolysis is determined.

The unsonicated and sonicated cultures of *L. delbrueckii subsp. bulgaricus* 11842 showed the highest lactase activity per gram of culture. Upon sonication, there was about 3 to 8 times increase in the enzyme activity. The optimum pH and temperature for incubation of the milk with the crude lactase isolated from *L. delbrueckii subsp. bulgaricus* 11842 was found to be 7.0 and 55°C. This is in keeping with the information shown in and discussed with respect to Figures 1 and 2. About 85% lactose hydrolysis was achieved in 16 h of incubation of sonicated culture of *L. delbrueckii subsp. bulgaricus* 11842 with milk as compared to about 25% lactose hydrolysis in control samples with unsonicated cultures. A slight drop in the pH of milk after incubation was observed as sonicated cultures contained 10^2-10^3 viable organisms. The slight drop in pH was not sufficient, however, to alter the taste of the product and in particular, the taste was normal without any hint of bitterness. it has also been found that the lactase as released from the sonicated microorganisms retains its activity at the higher incubation temperatures of, for example, 55°C. However, at this higher incubation temperature, the remaining rel ased contents which could be active in c nverting components of the milk are inactive at the higher incubation temperature of 55°C.

This may be due to the higher temperatures of incubation denaturing other proteins and/or destroying other radicals which could harm the milk quality during the incubation process.

Although not wishing to be bound by any theory, it is possible that the unexpected milk quality after lactose hydrolysis by incubation of the milk with the sonicated culture, is due to the higher incubation temperature inactivating proteins, other enzymes and other components except for the lactose. The results of the hydrolysis are set out in the following Table 1.

TABLE 1

Changes in pH, Enzyme Activity and Extent of Lactose Hydrolysis during Incubation of Milk with *L. delbrueckii subs. bulgaricus* 11842 Sonicated Culture

Time of Incubation (h)	pH	Enzyme Activity (U)*	Lactose Hydrolysis (%)
0	6.60	0.95	0
4	6.45	0.78	48
8	6.40	0.63	61
16	6.30	0.55	85

* U = μmole ONP/min.g culture

Example 9: **Effect of sonication on release of β-galactosidase**

Upon sonication, maximum lactase activity was achieved after 4 min of sonicating *L. delbrueckii subsp. bulgaricus* and *S. thermophilus*, and after 12 min of sonicating *L. acidophilus* cultures. High sonication time for *L. acidophilus* culture as compared with other bacterial cultures may be an indication of rigid cell wall of this organism. Onc the maximum lactase activity was achieved, there was no decrease in the enzyme activity on furth r sonication. This was in contrast with the observation of Kilara and Shahani (1976).

"Lactase activity of cultured and acidified dairy products". *J.Dairy Sci.*, who reported a decrease in enzyme activity after 7 min sonication of a yogurt culture. The decrease in enzyme activity in their study may have been due to an increase in temperature during sonication which would cause inactivation of the liberated enzyme, as observed in our experiments. Controlling temperature of the culture during sonicating is therefore a preferred aspect of the process in releasing lactase into the culture medium.

The lactase activity of the four bacterial cultures before and after sonication is shown in Table 2.

Table 2- Lactase activity of four bacterial cultures before and after sonication

	Lactase activity			
	Unsonicated		Sonicated	
Organisms	X	S.D.	X	S.D.
	(μmole o-nitro-phenol/min. g culture)			
L. delbrueckii subsp.bulgaricus	0.38	0.02	1.63	0.13
S.thermophilus	0.21	0.05	0.35	0.02
L.acidophilus	0.14	0.02	0.85	0.04

The unsonicated as well as sonicated cultures of L. delbrueckii subsp. bulgaricus showed the highest lactase activity per gram culture contained approximately the same number of organisms pre gram culture. Lactase produced by *S. thermophilus* and *L. delbrueckii subsp. bulgaricus* lactase are known as β-D-galactoside galactohydrolase (β-gal) (Wong et al, 1987). "Stimulation of rat growth by yogurt: A role of lactose and lactase". *Nutr. Reports International.* Upon sonication, there was about a 5-fold increase in the lactase activity of *L. delbrueckii subsp. bulgaricus* and

L. acidophilus, whereas *S. th rmophilus* exhibited only about a 1.5-fold increase in the lactase activity.

Sonication time to release β-gal was the highest for *L. acidophilus*; this organism also survived better than the others under acidic conditions, indicating the possibility of effective protection against adverse environment conditions such as found in the human gastrointestinal tract. However, *L. delbrueckii subsp. bulgaricus* possessed considerably more β-galactosidase activity than *L. acidophilus* or *S. thermophilus* especially in the skim milk system, and the survival in the acidic conditions was also satisfactory. Although *S. thermophilus* contained the highest total lactase activity in broth systems, its activity in skim milk was much less pronounced. Cultures of *S. cremoris* possessed negligible amount of β-gal activity under the present experimental conditions and thus the significance of its low pH survival for lactose malabsorbers needs to be studied further.

The process according to this invention, thereby, facilitates production of dairy products for lactose intolerant consumers, reasonable cost and allows a large segment of consumer population access to nutritious, economical food which have been denied in the past. The process of this invention does not introduce a foreign substance to the dairy products and hence, is safe and does not require regulatory approval.

The process of this invention may be used by the manufacturers of dairy products, or may be carried out by dairy producers. The technique is readily achieved, easy to use and reliable in the hydrolysis of lactose in dairy products.

Although preferred embodiments of the invention are described h rein in detail, it will be understood by those skilled in the art that variations may be made thereto without departing from the spirit of the inv ntion or the scope of the appended claims.

WE CLAIM:

1. A composition useful for enzymatically hydrolysing lactase in dairy products, said composition comprising a sonicated culture medium containing microbial cells of bacteria or yeast which produce lactase within the cells during culture wherein:

said cells are non-toxic to humans and compatible with dairy products;

sonication of said cells ruptures said cells to release thereby said lactase into said culture, and

said culture media containing ruptured cell wall material, any remaining whole cell material after sonication and contents of said ruptured cells.

2. A composition of claim 1 wherein said bacterial cells are selected from the group consisting of *Lactococcus, Lactobacillus, Leuconostoc, Streptococcus, Bifidobacterium, Propionibacterium, Pediococcus, Candida, Kluyveromyces and Sacharomyces*.

3. A process of enzymatically hydrolysing lactose in dairy products comprising introducing a composition of claims 1 or 2 into said dairy product and incubating said composition in said dairy product for a sufficient period of time to hydrolyse said lactose to an acceptable degree.

4. A process of claim 3 wherein said composition is introduced into milk.

5. A process of claim 3 wherein said incubation period is less than 24 hours.

6. A process of claim 3 wherein said incubation is carried out at a temperature in the range of 50° to 60°C.

7. A process of claim 3 wher in said incubation is carried out at a temperature of approximately 55°C and a pH of approximately 6.5.

8. A process for enzymatically hydrolysing lactose in dairy products comprising:

i) adding a culture of bacterial cells to said dairy product to form a dairy product mixture, said bacterial cells producing lactose in sufficient quantity to hydrolyse lactose in said dairy product, being non-toxic to humans and being compatible with said dairy product;

ii) sonicating said dairy product mixture for a sufficient period of time to rupture said bacterial cells and release thereby said lactase directly into said dairy product;

iii) incubating at 55°C and a pH of approximately 6.5 the in situ released lactase in said dairy product to hydrolyse said lactose to a sufficient degree; and

iv) processing, as required, said lactose reduced dairy product for end use.

9. A process of claim 8 wherein said bacterial cells are selected from the group consisting of *Lactococcus, Lactobacillus, Leuconostoc, Streptococcus, Bifidobacterium, Propionibacterium, Pediococcus*.

10. A process of claim 8 wherein said dairy product being treated is milk.

11. A process for preparing a composition of claim 1 comprising:

i) culturing said bacterial c lls in a suitable culture medium to produc lact se within said c lls and c ntinuing cultur f said c lls until lact se c ncentration is at a maximum f r harvest;

ii) sonicating said cultur medium to rupture a majority of said bacterial cells to release thereby said lactase into said culture medium; and

iii) processing said culture medium for storage and use.

12. A process of claim 11 wherein said culture medium is sonicated at a frequency of 16 KHz.

13. A process of claim 11 wherein said culture medium temperature is controlled during sonication to preserve lactase activity.

14. A process of claim 13 wherein said temperature is retained in the range of 20°C.

15. A process of claim 13 wherein sonication of said culture medium is carried out at pH 7.

16. A process of claim 16 wherein said bacterial cells are selected from the group consisting of *Lactococcus, Lactobacillus, Leuconostoc, Streptococcus, Bifidobacterium, Propionibacterium, Pediococcus*.

17. A process of claim 3 wherein said bacterial cells are of a species which, when treated by sonication to release cell contents into said culture medium and said medium is introduced to said dairy products and incubated at a temperature in the range of 50° to 60°C, the lactase retains lactose hydrolysing activity and other enzymes and proteins which could affect dairy product quality are neutralized.

18. A process of claim 17 wherein said dairy product is incubated at a pH in the range of 6.5.

19. A process of claim 18 wherein said dairy product is milk.

20. A process of claim 19 wherein said incubation temperature is in the range of 55°C.

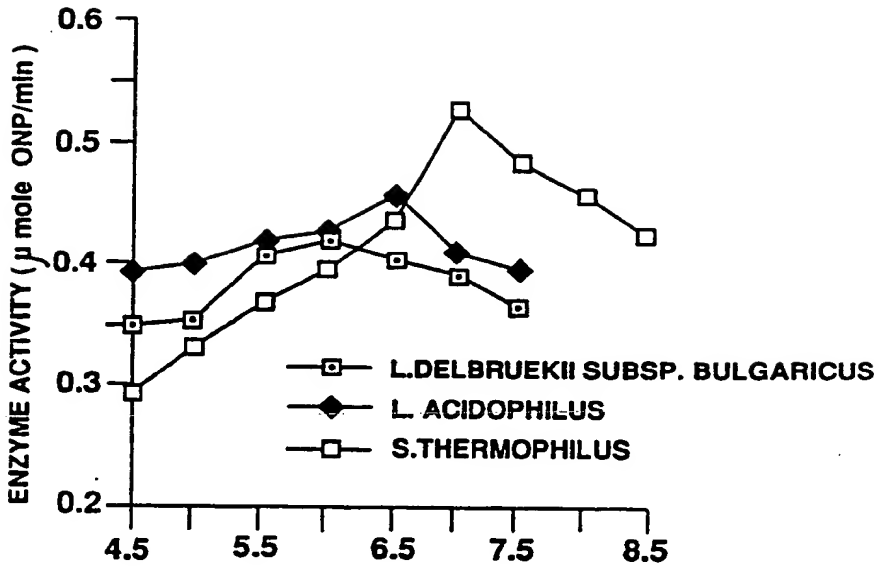

FIG.1.

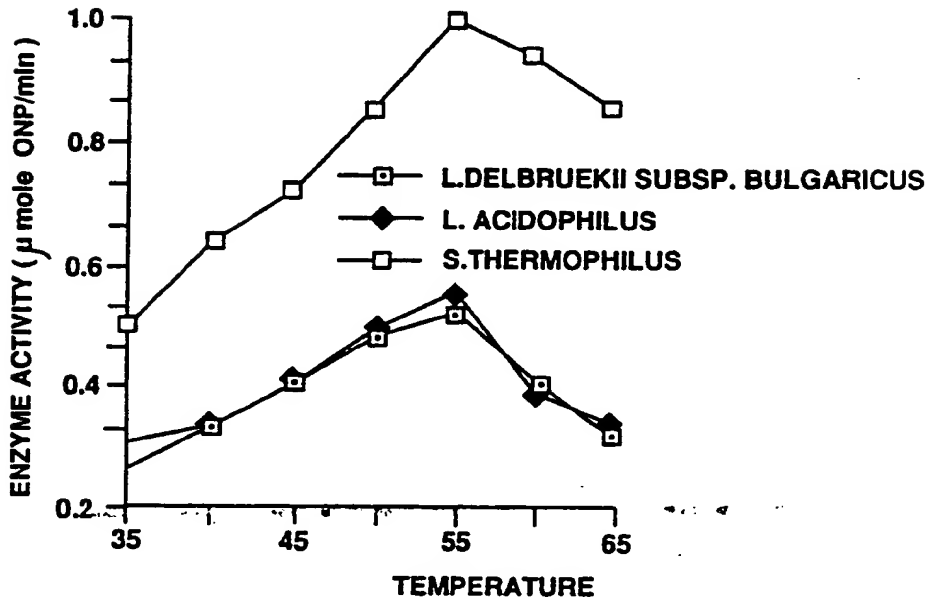

FIG.2.

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application No PCT/CA 92/00023

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N9/38; A23C9/12; //(C12N9/38; C12R1:225 C12R1:46C12R1:72C12R1:85)

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N ; A23C

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
P,X	MILCHWISSENSCHAFT. vol. 46, no. 9, 1991, MUNCHEN DE pages 570 - 573; N. SHAH ET AL.: 'Lactase activity and properties of sonicated dairy cultures' see page 570, left column, paragraph 2 - page 571, left column, paragraph 2 see page 571, right column, paragraph 2 - page 572, left column, paragraph 1 see page 572, right column, paragraph 3 - left column, last paragraph --- -/--	1-6,11, 13, 15-17, 19,20

* Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
16 MARCH 1992	26. 03. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	MONTERO LOPEZ B.

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	JOURNAL OF FOOD SCIENCE. vol. 55, no. 2, March 1990, CHICAGO US pages 506 - 509; N. SHAH ET AL.: 'Survival of Lactic Acid Bacteria and Their Lactases under Acidic Conditions' see page 506, right column, paragraph 2 -paragraph 6 see page 507, left column, paragraph 2 see page 507, right column, last paragraph - page 508, right column, paragraph 3 ---	1,2, 11-14,16
X	THE AMERICAN JOURNAL OF CLINICAL NUTRITION vol. 45, no. 3, 15 April 1987, USA pages 570 - 574; FRANK E. MCDONOUGH ET AL: 'Modification of sweet acidophilus milk to improve utilization by lactose-intolerant persons' see page 570, right column, paragraph 2 - page 571, left column, paragraph 1 see page 571, left column, paragraph 4; table I see page 574, left column, paragraph 2 -paragraph 3 ---	8,9